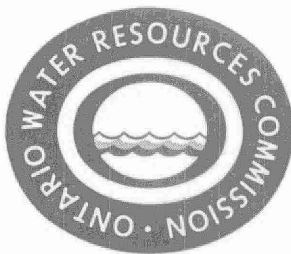


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LAKE EUTROPHICATION
A LABORATORY INVESTIGATION

DIVISION OF RESEARCH
ONTARIO WATER RESOURCES COMMISSION

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LAKE EUTROPHICATION
A LABORATORY INVESTIGATION

By

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Paper No. 2026

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Introduction

Eutrophication, the process by which lakes age and mature, is inherent to any body of water and results from the nutrient enrichment of that body of water whether from natural processes or as a result of man's activities. Natural processes may involve runoff from fallow land and forested areas and the erosion of river banks. Lake enrichment from man's activities may result from farm runoff and drainage, sewage and industrial effluents and urban runoff. Man's activities have greatly increased the rate of eutrophication, resulting in premature nuisance levels of algae and other plant life in many of our lake systems.

The general symptoms of eutrophication are the increased fertility of the water with a resultant increase in primary productivity leading, in the most severe cases to "blooms" of algae. Large masses of algae cause wide daily fluctuations in oxygen content of the water and when this algae dies and decomposes it imposes a great demand upon the oxygen content of the receiving water. Such conditions can cause death to fish and the production of foul odours with many subsequent economic consequences.

The nutrient requirements of algae have been the subject of considerable research effort in many disciplines throughout the world. This paper deals with the author's personal investigations of a singular phase of the nutrient enrichment problem and no attempt has been made to present a literature review of other works. For references on other works, the reader is referred to Mackenthun's "Nitrogen and Phosphorus in Water. An annotated bibliography of their biological effects", U.S. Department of Health, Education and Welfare, PHS, Pub. No. 1305, 1965.

Past criteria for sewage effluent disposal to fresh water systems have generally been limited to Biochemical Oxygen Demand, (BOD), Suspended Solids (SS) and coliform bacteria with objectives set at 15 ppm, 15 ppm and 2,400 per 100 ml. respectively. Effluent polishing facilities have recently been installed at many locations to further reduce BOD and SS concentrations in the effluent.

In spite of this advance towards increasing BOD and SS removals of sewage treatment plant effluents, eutrophication is continuing to proceed at an alarming rate as evidenced by the increasing frequency and severity of algal blooms recently appearing in our resort area lakes. Plant nutrients, particularly phosphorus, nitrogen and carbon,

are considered the leading substances contributing to this eutrophication. Sanitary engineers and biologists are now calling for nutrient removal as an integral part of conventional waste treatment.

Present technology of sewage treatment is such that the degrees of treatment available are virtually unlimited, ranging from plain sedimentation to complete renovation. There is a diversity of opinion however, as to the relative importance of the various nutrient materials in causing high levels of algal production so often experienced in our lakes and rivers and controversy exists over which nutrients should be removed.

While laboratory model scale nutrient removal studies were being carried out, it was felt expedient to study the relative effectiveness of various advanced methods of sewage treatment in reducing the rate of eutrophication of lakes required for the final disposal of municipal sewages. To this end, simulations were made of a soft water lake, in its initial stages of eutrophy, typical of the Muskoka area. A comparison was made of the effects of various effluents on stimulating algal growth in the simulated "lakes" under controlled conditions of light and temperature.

This paper outlines the results of this particular study.

Simulated "Lake" Operation

Water from Gull Lake at Gravenhurst, Ontario, was acclimatized in an open container in the laboratory for 6 days and then seeded 5 mls. to 175 litres from a laboratory culture of naturally occurring algae and allowed to stand for an additional 6 days. The water was then decanted out of the container, thoroughly mixed and poured into four lake cultures. These cultures, each constructed of 3/8" plexiglass and 35 litres capacity, were placed in a growth chamber of controlled conditions of light and temperature and further acclimatized for an additional 2 week period.

At the end of the acclimatization period, a comparison of extensive chemical analyses and algae determinations on each culture indicated no appreciable difference in quality among them. Very low concentrations of the following algae were observed in each culture:

Ankistrodesmus sp., Asterionella sp., Chlorella sp., and several transparent forms. Average values of the chemical analyses are presented in Table 1.

The four cultures were then fed, on a daily draw and fill basis, with a 1.75 litre mixture of 40% distilled water, 40% Gull Lake water (collected once a week and refrigerated until use) and 20% treated sewage. The

sewage effluents used will be described in the following section. The feed mixtures were made to represent a combination of natural drainage and municipal effluent discharges to the lake.

Culture No. 1
after 28 days



Collecting Gull Lake
Water Samples

Table 1

Chemical Analyses of Gull Lake Water

	<u>mg/l</u>
Total Phosphorus (as P)	0.010
Orthophosphorus (as P)	0.006
Ammonia Nitrogen (as N)	0.18
Total Kjeldahl Nitrogen (as N)	0.84
Nitrate Nitrogen (as N)	0.03
Total Carbon (as C)	10.0
Inorganic Carbon (as C)	2.0
Alkalinity (as CaCO ₃)	9
Total Iron (as Fe)	10.10
Suspended Solids	2
Dissolved Solids	43
Hardness (as CaCO ₃)	18
Dissolved Silica (as SiO ₂)	1.7
pH	7.2

The feed mixtures were fed into each of the cultures so as to give a 20-day detention period in each. Displaced water was used without filtering for chemical and biological determinations. Any evaporation from the culture was made up with distilled water. Daily feeding of the cultures was continued for 28 days until a markedly noticeable difference was observed between the lake cultures.

Throughout the study, the growth chamber temperature was controlled at $21^{\circ}\text{C} \pm 1.5$. Lighting was time clock controlled such that for 8 hours the cultures received no light. This was followed by a 2-hour period at 2,000 foot-candles at the liquid surface, followed in turn by a 12-hour period at 4,000 foot-candles, and a further 2-hour period again at 2,000 foot-candles.

Culture Feed Solutions

The sewage effluents used for the feed make-up of the four cultures were as follows:

1. Filtered conventional activated sludge effluent,
2. Raw sewage chemically treated for phosphorus removal,
3. The effluent of No. 2 followed by ion exchange for ammonia removal, and
4. The effluent of No. 3 followed by carbon filtration for carbon removal.

The activated sludge effluent for No. 1 was obtained from an operating municipal sewage treatment plant, the raw sewage of which was used to prepare the other three effluents from laboratory scale systems.

Effluent No. 2 was that of a model chemical treatment plant employing lime and ferric chloride addition to a combined flocculation-sedimentation tank system as previously described in OWRC Research Publication No. 36.

The effluent No. 3 was made up by passing No. 2 effluent through a 30" x 4" diameter ion exchange column packed with the Dowex resin, CCR-1.

No. 4 effluent was made up by passing the effluent of No. 3 through a 24" x 3" diameter carbon column packed with granular activated carbon.

Experimental rates were used through both columns to obtain maximum ammonia and carbon removals.

It should be noted that the ion exchange resin used for ammonia removal was in its hydrogen form and therefore, as well as removing ammonium ions, the cations associated with bicarbonate ion were also readily exchanged with the hydrogen ions of the resin. This resulted in the removal of alkalinity from the water justifying the low inorganic carbon content of culture No. 3 feed solution of Table 2.

Comparing culture No. 3 feed solution to that of No. 4, it appears, at first, that the carbon column was ineffective in removing carbon. However, the reason for the low inorganic carbon content of No. 3 has just been explained and it can be seen that the organic carbon of No. 4 averaged only 3.2 mg/l, compared to 9.2 mg/l for No. 3. Thus, the carbon column was quite effective in removing the dissolved organic material from the chemical plant effluent.

Fresh samples of each of these effluents were collected once a week and refrigerated until used. There were no appreciable changes in measured parameters within the samples during storage and no appreciable differences in these parameters from week to week except for the conventional activated sludge effluent.

Average chemical analyses for each of the culture feed solutions, after the addition of the sewage effluent to the Gull Lake water and distilled water, are presented in Table 2. Table 3 presents the four weekly analyses of phosphorus, nitrogen and carbon parameters of the feed solutions for Culture No. 1 made up with the activated sludge effluent.

Table 2
Chemical Analyses of Feed Solutions

	<u>Culture No.</u>			
	1	2	3	4
Total Phosphorus	1.62	0.08	0.08	0.07
Orthophosphorus	1.17	0.02	0.07	0.01
Ammonia Nitrogen	4.2	3.8	0.06	0.07
Total Kjeldahl Nitrogen	4.7	6.8	2.2	0.84
Nitrate Nitrogen	0.29	0.29	0.16	0.20
Total Carbon	21.0	17.8	10.2	10.2
Inorganic Carbon	13.5	6.5	1.0	7.0
Alkalinity	66.5	27.0	0.0	51.8
Total Iron	0.06	0.10	0.08	0.06
Total Solids	158	250	60	262
Suspended Solids	1	2	1	2
Dissolved Solids	156	230	58	260
Hardness	61	66	15	160
Dissolved Silica	3.2	1.7	1.6	2.0

All Units are mg/l.

Table 3

Weekly Analyses of Feed Solutions for Culture No. 1

	<u>Week Number</u>			
	1	2	3	4
Total Phosphorus	2.28	1.70	1.37	1.14
Orthophosphorus	1.79	1.40	1.21	0.32
Free Ammonia	4.7	4.2	4.3	3.4
Total Kjeldahl Nitrogen	5.6	4.6	4.4	4.2
Nitrate Nitrogen	0.35	0.24	0.24	0.34
Total Carbon	23	22	24	15
Inorganic Carbon	16	14	16	8

All Units are mg/l.

Culture Results

After 7 or 8 days of feeding the four lake cultures it became visually apparent that Culture No. 1, receiving the filtered conventional activated sludge effluent mixture, was much more productive than the other three. Algae counts of all four cultures over this period showed increased productivity in Culture No. 1 only, with no measurable increase in any of the other three cultures, (4600 asu/ml for No. 1 as compared to an average of 60 for cultures 2, 3 and 4).

Daily feeding was continued until, after a total period of 28 days, Culture No. 1 took on the appearance of a highly productive sewage lagoon, while the three remaining cultures showed no appreciable productivity. Feeding of the four cultures was therefore discontinued.

Figures 1, 2 and 3 present time graphs of several parameters for Culture No. 1 only.

Supporting Studies

Since there was no appreciable algal production in either of Cultures 2, 3 or 4 during the feeding period, it was felt that perhaps the lime or ferric chloride used in the chemical treatment process had some inhibitory

effect on algal growth. Three 250 ml erlenmeyer flasks were filled to the 200 ml level with water from Culture No. 2 for further algal growth studies and designated as 2 (a), 2 (b) and 2 (c). Two of these cultures were fed with disodium hydrogen phosphate (Na_2HPO_4), with 2 (a) receiving 0.032 mg. as P and 2 (b) receiving 0.072 mg. as P bringing their orthophosphate concentrations to 0.17 and 0.33 mg./l, respectively. Flask 2 (c) was maintained as a control.

The three flasks were placed in the growth chamber and observed over a four week period. At the same time the three original lake Cultures 2, 3 and 4 were inoculated at a ratio of 1 ml. to 85 l, with algae from Culture No. 1 and also observed for a further four week period with no feed addition.

Observations of the three flasks over the four week period showed considerable algal growth in both flasks to which the phosphorus was added. There was no increased productivity in the control flask or in either of the three reseeded original lake cultures. Algal counts made on the mixed contents of the three flasks at the end of the four week period indicated slightly greater algal production in

the flask to which the higher dose of phosphorus was added (4,820 asu/ml as compared to 4,200 asu/ml). In all cases the predominant algae were of the Chlorella species.

Further supporting studies were also carried out on Cultures 3 and 4. Three 200 ml erlenmeyer flasks of each were collected and designated as 3 (a), (b) and (c), and 4 (a), (b), and (c). Flasks 3 (c) and 4 (c) were kept as controls while chemical additions were made to each of the other four. 3 (a) and 3 (b) were adjusted to a pH of 6.8 by the addition of NaOH and 3 (a) was fed with 4 mg. NaHCO_3 and 0.005 mg as P of Na_2HPO_4 , bringing the alkalinity and orthophosphate levels to 12 and 0.045 mg/l, respectively. 3 (b) was fed with 0.01 mg as P of Na_2HPO_4 bringing its orthophosphate level up to 0.07 mg/l.

Flask 4 (a) was also fed with 4 mg NaHCO_3 and 0.005 mg as P of NaHPO_4 raising its alkalinity and orthophosphate levels to 48 and 0.035 mg/l, respectively. 4 (b) received 0.01 mg as P of Na_2HPO_4 with a resultant orthophosphate concentration of 0.06 mg/l. These additions are all presented in Table 4.

All flasks were kept in the growth chamber and observed for algal growth over a period of four weeks, at the end of which time algal counts were made on all flasks. The results of these counts have also been presented in Table 4.

Table 4

Chemical Additions to, and Algal Counts of 200 ml. Flasks
of Cultures 2, 3 and 4

<u>Flask</u>	<u>Material Added</u>	<u>Algae Count asu/ml</u>
2 (a)	0.032 mg P	4200
2 (b)	0.072 mg P	4800
2 (c)	Control	12
3 (a)	NaOH to pH 6.8 4 mg NaHCO ₃ 0.005 mg P	3624
3 (b)	NaOH to pH 6,8 0.01 mg P	2177
3 (c)	Control	7
4 (a)	4 mg NaHCO ₃ 0.005 mg P	118
4 (b)	0.01 mg P	38
4 (c)	Control	9

As can be seen from Table 4, Flasks 3 (a) and 3 (b) supported considerable growth while Flasks 4 (a) and 4 (b) supported only a small amount of growth. Again, Chlorella was the predominant species. Both control flasks supported insignificant growth.

Data Interpretation

As this was a controlled laboratory investigation, the direct application of the results of this study to actual field conditions is not warranted. The study does, however, present a comparison of the effects of variously treated sewage effluents on the eutrophication of receiving waters and as such, an estimation of the effects of such effluents on receiving water eutrophication under field conditions is now possible.

With this view in mind the following interpretation of the study information is presented.

Figures 1, 2 and 3, derived from the first phase of the experiment, quite strikingly suggest the roles of phosphorus, nitrogen and carbon in the algal productivity of a lake.

a) Phosphorus

Figure 1 shows the relationship of ortho- and total phosphorus to the growth of algae. Initially, until

the increase in growth rate of the algae occurred, the actual concentrations of ortho- and total phosphorus followed closely that of the added values (the values theoretically accumulated in the "lakes" due to the input). Following the 6th day, however, there was a rapid decrease in orthophosphorus concentration as it was being assimilated by the algae growth. The total phosphorus concentration also dropped off from the added concentration as the algae began to settle out on the bottom.

b) Nitrogen

Figure 2 shows the relationship of various nitrogen forms to the growth of algae. As can be seen the actual free ammonia increased in accordance with the feed until the rapid algal growth rate occurred on about the 6th day. Following the 6th day there was a rapid decrease in the free ammonia content of the culture water in spite of its relatively high input and it was maintained at a low level until the end of the study period. Nitrate nitrogen continued to build up until the ammonia was reduced to a low concentration and then it also was utilized. This would indicate that free ammonia is more readily assimilated by the algae than nitrate although at low levels of ammonia the algae will use any available nitrate.

Looking at the two graphs of total nitrogen it can be seen that in the latter stages of the study the actual level of nitrogen began to exceed the added values until by the 28th day the actual content more than doubled (226 mg as compared to 109 mg) the added nitrogen. This would imply a supplementary source of nitrogen towards the end of the study through nitrogen fixation from the atmosphere, either by the algae themselves or through the symbiotic activity of bacteria and algae. Throughout the study Chlorella was by far the predominant species of algae but during the final week there was a rapid increase in a filamentous type of algae, identified tentatively as a species of Spirogyra; both are believed to be non-nitrogen fixers.

(c) Carbon

A similar set of graphs in Figure 3, indicates the relationship of carbon to algal growth in the lake culture. Comparing the graph for the added total carbon with that of the actual total carbon, there is an unexplained drop in the actual level during the initial period of high algal growth rate. However, by the 17th day the actual level began to exceed the added level indicating a supplementary source of carbon also through the direct assimilation of atmospheric CO₂.

The graph giving the actual level of inorganic carbon shows an initial build-up followed by a progressive depletion following the increased algal growth. This was again followed by a slow build-up of inorganic carbon which corresponded to the period when CO₂ assimilation began occurring. During the period of high algal growth rate the actual organic carbon build-up considerably exceeded that of the added level, reflecting the conversion of inorganic carbon in algal synthesis. A drop in the rate of organic carbon increase occurred in the latter stages of the study due in part to the sedimentation of algae and also to the decreased carbon content of the feed.

Supporting Studies

Table 5 presents a more detailed description of the supporting studies. A comparison of the phosphorus, nitrogen and carbon contents of the flask cultures are presented as well as algal concentration levels at the conclusion of the four week test period. It should perhaps be pointed out that only one single algal count was made of each flask at the end of the four week period. Thus, the presented algal concentrations for the supporting studies do not necessarily compare the rates of algal growth or the maximum concentrations obtained.

Looking at No. 2 (a), (b) and (c), the only variable between the three flasks is the phosphorus content added in the form of disodium hydrogen phosphate, an ortho-phosphate form. As can be seen, both 2 (a) and 2 (b) supported an appreciable growth. As the only difference between the three cultures was the phosphorus content, it must have been phosphorus that was limiting in 2 (c), therefore, the limiting phosphorus concentration in this case was somewhere above 0.013 mg/l total phosphorus. If there can be considered a significant difference between 4,200 and 4,820 asu/ml algal concentration, it would appear that the higher the phosphorus concentration, at least up to 0.33 mg/l, the greater the algal productivity.

Comparing now 3 (a), (b) and (c) it would appear that phosphorus was again limiting in 3 (c). Since 3 (a) had significantly greater growth than 3 (b) and yet its phosphorus concentration was lower than that of 3 (b) it would appear that at a concentration of phosphorus above 0.018 mg/l, carbon became the controlling factor for algal growth. It would appear therefore, that a carbon content of 8 mg/l or less will exert a partially limiting effect on algal growth. Comparing 3 (a) with 2 (c), we see that the limiting phosphorus concentration lies somewhere between 0.013 and 0.018.

Looking now at 4 (a), (b) and (c) we can assume no significant difference in algal productivity between the three. Comparing 4 (b) with 3 (a), however, would indicate that nitrogen was the limiting factor to algal growth in 4 (b) with the limiting concentration lying somewhere between 0.32 and 0.53 mg/l of inorganic nitrogen. It should be mentioned that although nitrite nitrogen determinations were made throughout the entire study, all nitrite concentrations were insignificant being less than 0.005 mg/l.

If we assume from 3 (b) that the limiting carbon concentration is about 5 mg/l, from 4 (b) that the limiting nitrogen concentration is 0.35 mg/l and from 2 (c) that the limiting phosphorus concentration is 0.015 mg/l, we can establish a limiting C:N:P ratio of 100:7:0.3.

Table 5
Supporting Study Data

		#2			#3			#4		
		(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)
Total Phosphorus	mg/l as P	0.17	0.33	0.013	0.018	0.038	0.013	0.011	0.031	0.006
Orthophosphorus	mg/l as P	0.16	0.32	0.003	0.011	0.031	0.006	0.008	0.028	0.003
Free Ammonia	mg/l as N	2.9	2.9	2.9	0.36	0.36	0.36	0.18	0.18	0.18
Nitrate	mg/l as N	0.26	0.26	0.26	0.17	0.17	0.17	0.14	0.14	0.14
Total Carbon	mg/l as C	14.0	14.0	14.0	10.85	8.0	8.0	15.85	13.0	13.0
Inorganic Carbon	mg/l as C	5.0	5.0	5.0	3.58	1.0	1.0	10.58	8.0	8.0
Algae Conc.	asu/ml	4200	4820	12	3624	2177	7	118	38	9

Conclusions

Keeping in mind the limitations of this study, the following conclusions are presented:

- 1) In its present state of eutrophy both nitrogen and phosphorus are limiting to algal growth in Gull Lake, a typical soft-water lake of the Muskoka area. Carbon levels are such that they would support a moderate growth of algae,
- 2) Phosphorus levels in excess of 0.015 mg/l as P, and nitrogen levels in excess of 0.5 mg/l as N are required to produce an algal growth response in the water under study,
- 3) Free ammonia is used by Chlorella in preference to nitrate nitrogen, but nitrate is readily assimilated when the free ammonia has been depleted. Once algal growth is established, and under favorable phosphorus conditions, both carbon and nitrogen may be obtained from the atmosphere for algal growth,
- 4) At levels below 8 mg/l, carbon is partially limiting to algal growth and exerts more of an influence on algal productivity than increased phosphorus.
- 5) For Gull Lake, chemical treatment of sewage for phosphorus removal will greatly reduce the algal growth response to that sewage as compared to conventional activated sludge treatment.

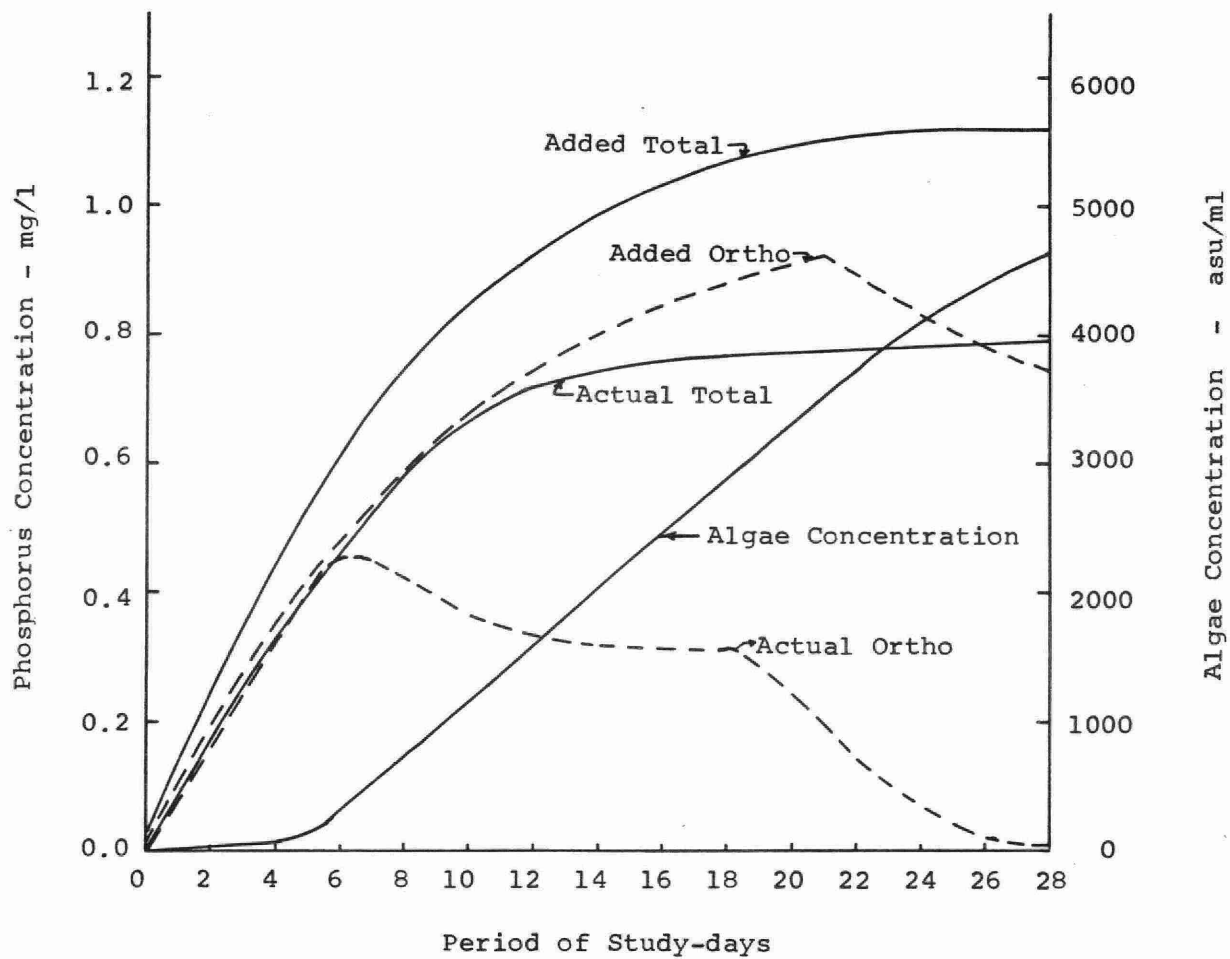


FIGURE 1: Phosphorus.

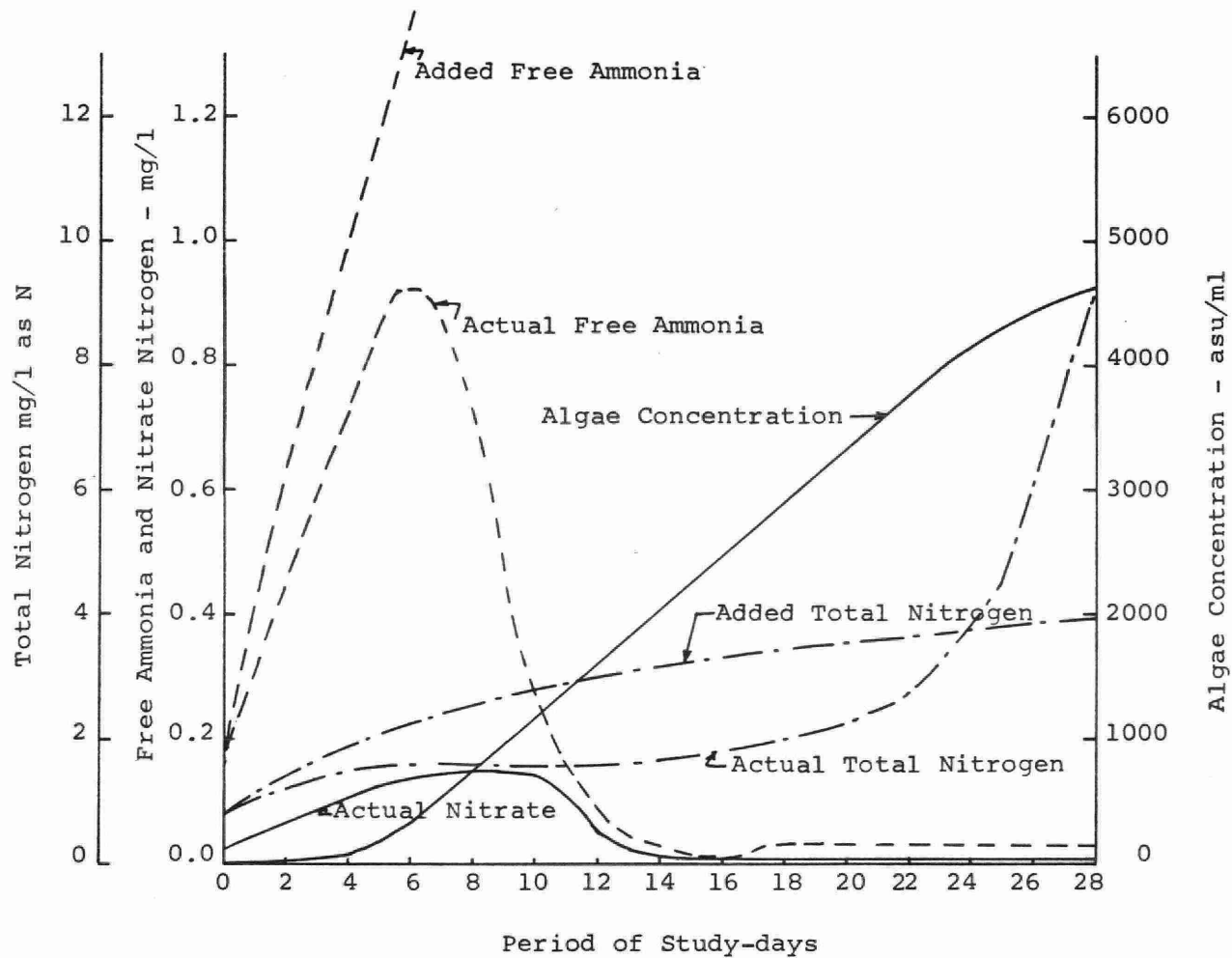


FIGURE 2: Nitrogen.

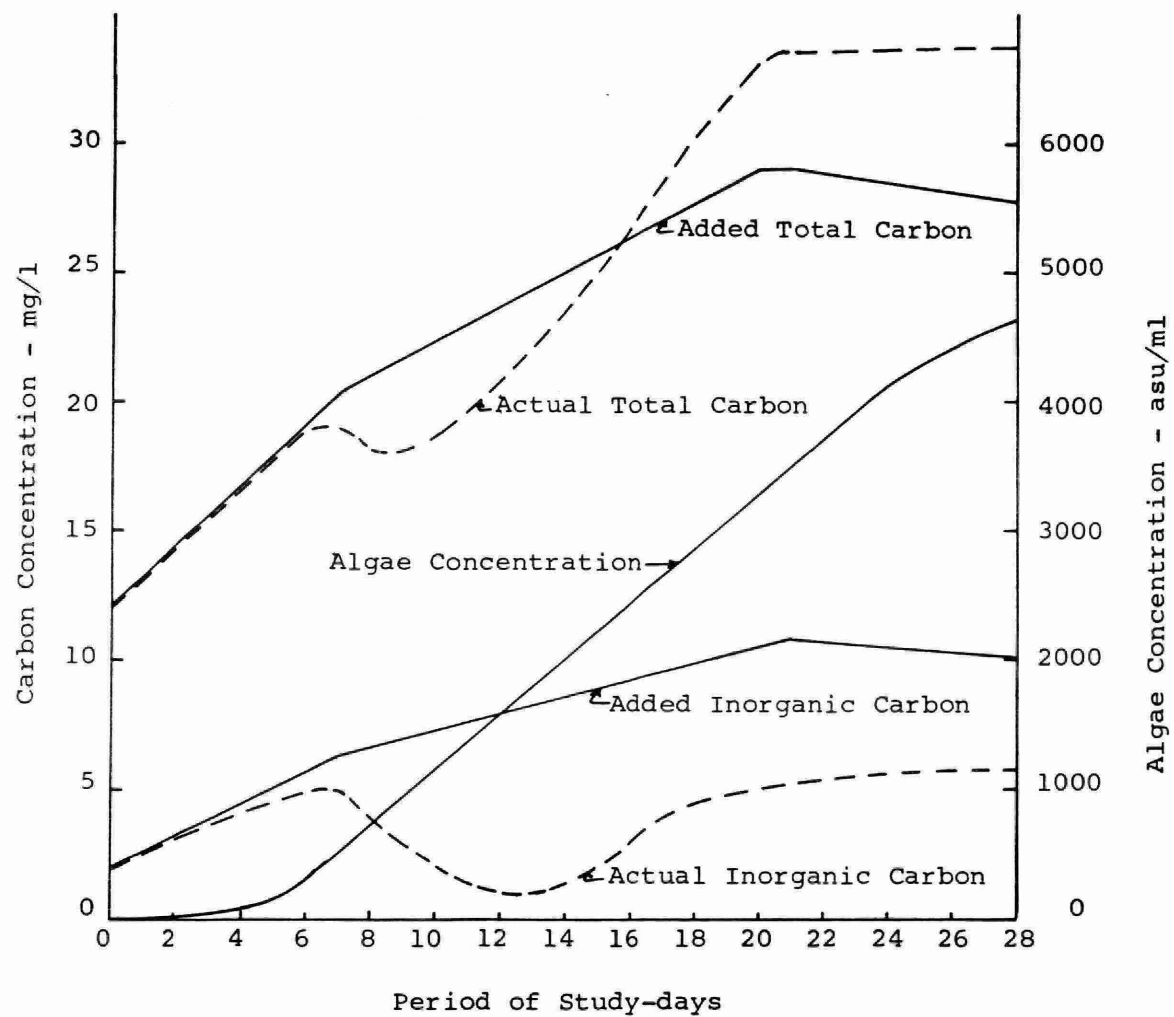


FIGURE 3: Carbon.

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